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Investigation of Antioxidant Activity of 3, 5-Dimethoxyaniline Derivatives

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ABSTRACT

*A series of new 3,5-dimethoxyaniline analogous, **3a-g** were prepared and their antioxidant activity was determined. The chemical structure was confirmed by UV-visible, FT-IR and ^{1}H NMR spectral studies. The structure-activity relationship (SAR) for antioxidant activity was discussed. All the compounds showed DPPH radical scavenging activity, where compounds **3b**, **3c** and **3d** were the best radical scavengers.*

Keywords: 3,5-Dimethoxyaniline, AldehydesDPPH, Antioxidant.

INTRODUCTION

In recent years, there has been an increased interest in the application of antioxidants to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. Free radicals play a role in the pathogenesis of chronic degenerative diseases including cancer, autoimmune, inflammatory, cardiovascular and neurodegenerative diseases and aging [1-4]. It is also known that oxidative stress can be induced by a wide range of environmental factors including UV stress, pathogen invasion, herbicide action and oxygen shortage [5]. Owing to these facts, synthetic and natural compounds with potential antioxidant activity are receiving increased attention in biological research, medicine and pharmacy [6].

Schiff bases are characterized by the imine group which is important in elucidating the mechanism of transamination and racemisation reactions in biological systems [7]. Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behaviors have been studied [8]. They have been synthesized from a variety of compounds such as amino thiazoles, 2-hydroxy-1-napthalaniline, amino sugars, aromatic aldehydes, ketones, isatin, triazole ring, thiosemi carbazides, amino acids and pyrazolone [9, 10]. Antibacterial, antifungal, antitumor and anticancer activities of some Schiff bases have been reported and they are active against a wide range of organisms [11]. Some Schiff bases bearing aryl groups or heterocyclic residues possessing excellent biological activities have attracted the attention of many researchers in recent years [12]. The Schiff bases formed from aromatic aldehydes, ketones and their derivatives are quite stable. Many Schiff bases are known to be medicinally important and are used to design medicinal compounds [13]. Treatment of 1,2-indanedione with 3,5-dimethoxybenzenamine in benzene afforded several products have been reported [14]. Synthesis of poly(2,5-dimethoxyaniline) and poly(aniline-Co-2,5-dimethoxyaniline) has been reported [15]. Crystal

structure of some novel compounds from 3,5-dimethoxyaniline have been reported [16]. In this respect, the present paper reports the preparation and antioxidant activity of a new class of Schiff bases, **3a-g**.

MATERIALS AND METHODS

All solvents and reagents were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., India**.** Melting points were determined using SELACO-650 hot stage melting point apparatus and were uncorrected. Elemental analyses were recorded on VarioMICRO superuser V1.3.2 Elementar. The UV spectra were recorded using Analytik Jena Specord 50 UV–Vis spectrophotometer. FT-IR spectra were recorded using a Jasco FTIR-4100 series. ^{1}H-NMR spectra were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO-d_6 as a solvent and TMS as internal standard (chemical shift in δ ppm). All the compounds **3a-g** was prepared according to the reported procedure [17].

General procedure for the preparation of 3,5-dimethoxyaniline derivatives 3a-g: Equimolar concentrations of different aldehydes (0.003 mol) and 3,5-dimethoxyaniline (0.003 mol) were stirred for 4-6 h at room temperature in absolute ethanol (25 mL) and then 2-3 drops of concentrated sulfuric acid was added to the mixture. The progress of the reaction was monitored by TLC until the reaction was complete. It was cooled to 0 °C, and the precipitate was filtered, washed with diethyl ether and the residue was recrystallized from methanol.

N-(2-Chloro-6-fluorobenzylidene)-3,5-dimethoxybenzenamine (3a): The general experimental procedure described above afforded **3a**, and the product obtained from 3,5-dimethoxybenzenamine (**1**) (0.50 g) and 2-chloro-6-fluorobenzaldehyde (**2a**) (0.47 g). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.65 (s, 1H, HC=N), 7.42-7.40 (m, 3H, Ar-H), 6.53-7.12 (s, 3H, Ar-H), 3.31 (s, 6H, OCH_3). Anal. Calcd. for $C_{15}H_{13}ClFNO_2$: C, 61.34; H, 4.46; N, 4.77; Found: C, 61.22; H, 4.58; N, 4.56 %.

2-((3,5-Dimethoxyphenylimino)methyl)phenol (3b): The general experimental procedure described above afforded **3b**, and the product obtained from 3,5-dimethoxybenzenamine (**1**) (0.50 g) and 2-hydroxybenzaldehyde (**2b**) (0.37 mL). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.60 (s, 1H, HC=N), 7.921-7.86 (m, 4H, Ar-H), 6.61-7.10 (s, 3H, Ar-H), 6.42 (s, 1H, OH), 3.34 (s, 6H, OCH_3). Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44; Found: C, 70.25; H, 5.78; N, 5.56 %.

4-((3,5-Dimethoxyphenylimino)methyl)-2-methoxyphenol (3c): The general experimental procedure described above afforded **3c**, and the product obtained from 3,5-dimethoxybenzenamine (**1**) (0.50 g) and 4-hydroxy-3-methoxybenzaldehyde (**2c**) (0.46 g). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.68 (s, 1H, HC=N), 7.97 (s, 1H, Ar-H), 7.84 (d, 1H, Ar-H), 7.75 (d, 1H, Ar-H), 6.61-7.11 (s, 3H, Ar-H), 6.54 (s, 1H, OH), 3.35 (s, 9H, OCH_3). Anal. Calcd. for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88; Found: C, 66.72; H, 5.78; N, 4.66 %.

4-((3,5-Dimethoxyphenylimino)methyl)phenol (3d): The general experimental procedure described above afforded **3d**, and the product obtained from 3,5-dimethoxybenzenamine (**1**) (0.50 g) and 4-hydroxybenzaldehyde (**2d**) (0.37 g). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.53 (s, 1H, HC=N), 7.94 (d, 2H, Ar-H), 7.44 (d, 2H, Ar-H), 6.72-7.22 (s, 3H, Ar-H), 6.42 (s, 1H, OH), 3.31 (s, 6H, OCH_3). Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44; Found: C, 70.25; H, 5.78; N, 5.36 %.

N-(4-Ethoxybenzylidene)-3,5-dimethoxybenzenamine (3e): The general experimental procedure described above afforded **3e**, and the product obtained from 3,5-dimethoxybenzenamine (**1**) (0.50 g) and 4-ethoxybenzaldehyde (**2e**) (0.41 mL). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H, HC=N), 8.44 (d, 2H, Ar-H), 7.99 (d, 2H, Ar-H), 7.85 (s, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 4.13 (q, 2H, CH_2),2.92 (s, 6H, OCH_3), 2.36 (t, 3H, CH_3). Anal. Calcd. for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91; Found: C, 71.75; H, 6.78; N, 4.76 %.

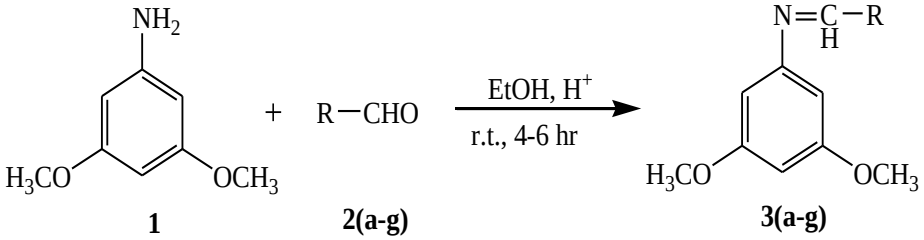
(3,4-Dimethoxy-benzylidene)-(3,5-dimethoxy-phenyl)-amine (3f): The general experimental procedure described above afforded **3f**, and the product obtained from 3,5-dimethoxyaniline (**1**) (0.50 g) and 3,4-dimethoxybenzaldehyde (**2f**) (0.50 g). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.40 (s, 1H, HC=N), 7.10 (d, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 6.75 (d, 1H, Ar-H), 6.28 (s, 3H, Ar-H), 3.64 (s, 12H, OCH_3). Anal. Calcd. for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65; Found: C, 67.85; H, 6.28; N, 4.46 %.

(3,5-Dimethoxy-phenyl)-(3,4-dimethyl-benzylidene)-amine (3g): The general experimental procedure described above afforded **3g**, and the product obtained from 3,5-dimethoxyaniline (**1**) (0.50 g) and 3,4-dimethylbenzaldehyde (**2g**) (0.40 g). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.42 (s, 1H, HC=N), 7.12 (d, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 6.81 (d, 1H, Ar-H), 6.26 (s, 3H, Ar-H), 3.60 (s, 6H, OCH_3), 2.30 (s, 6H, CH_3). Anal. Calcd. for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.11; N, 5.20; Found: C, 75.85; H, 7.28; N, 5.36 %.

Antioxidant activity: The free radical scavenging activity of the compounds was studied *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay method [18]. Stock solution of the drug was diluted to different concentrations in the range of 100-200 µg mL^{-1} in methanol. Methanolic solution of the compounds (2 mL) was added to 0.003 % (w/v) methanol solution of DPPH (1 mL). The mixture was shaken vigorously and allowed to stand for 30 min. Absorbance at 517 nm was determined and the percentage of scavenging activity was calculated. Ascorbic acid was used as the standard drug. The inhibition ratio (I %) of the tested compounds was calculated according to the following equation: *I % = (Ac-As) / Ac × 100*, where *Ac* is the absorbance of the control and *As* is the absorbance of the sample. The concentration of compounds providing 50 % scavenging of DPPH$^{\cdot}$ (IC_{50}) was calculated from the plot of percentage inhibition against concentration (µg mL^{-1}). All tests and analyses were done in triplicate and the results were averaged.

RESULTS AND DISCUSSION

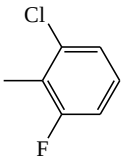
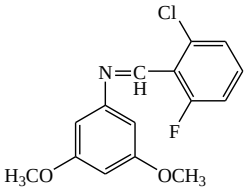
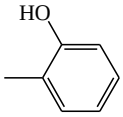
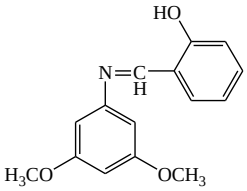
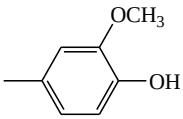
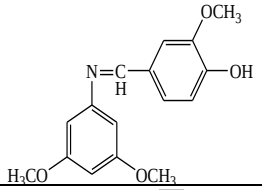
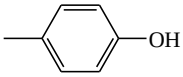
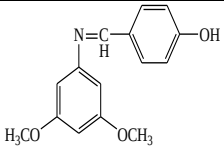
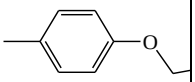
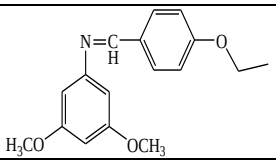
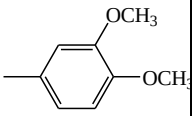
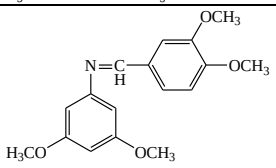
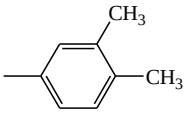
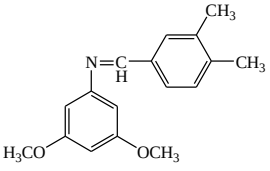
The reaction of 3,5-dimethoxyaniline (**1**) with different aldehydes, were carried out in the presence of ethanol as solvent with a good yield ranging from 70- 82 %. Compounds were characterized by UV-Visible, FT-IR and ^{1}H NMR spectral studies. The chemical structures and physical data of all the compounds are given in table 1. The electronic absorption spectra of the compounds showed new bands, and the appearance of longer wavelength absorption band in the UV-visible region confirms the formation of compounds. The synthetic route of the compounds is outlined in scheme 1.



1 **2(a-g)** **3(a-g)**

Scheme 1

Table-1: Chemical structures and physical data of **3a-g**

Compound	R	Structure	Mol. Wt.	Yield (%)	UV-visible	M.R (°C)
3a			293.7	70.0	450	107-109
3b			257.3	72.4	410	102-104
3c			287.3	79.0	460	105-107
3d			257.3	82.0	397	110-112
3e			285.1	78.4	373	116-118
3f			301.4	78.4	398	156-158
3g			269.3	80.2	425	128-130

The absence of NH_2 and C=O absorption bands in the IR spectra confirmed that the compounds were obtained. The appearance of a medium to strong absorption band at around 1600 cm^{-1} is due to the stretching vibration of C=N bond formation in the Schiff base compounds. IR spectral data of **3a-g** was depicted in Table 2. The proton spectral data agree with respect to the number of protons and their

chemical shifts with the proposed structures. The proton spectral data of **1** shows resonance at δ 5.45 ppm (s, 2H, NH_2). In all the new compounds, the above resonance disappeared and additional resonances assigned to the -CH=N- (δ 8.81 – 8.40 ppm) was observed, which confirmed the products.

Table 2: IR data of Schiff bases **3a-g**

Compound	N-H	O-H	Aromatic C-H	C=N	C-F	C-O	C-Cl
1	3392	-	3064	-	-	-	-
3a	-	-	3081	1601	1302	1155	722
3b	-	3419	3082	1606	-	1156	-
3c	-	3420	3061	1615	-	1153	-
3d	-	3447	3075	1600	-	1158	-
3e	-	-	3065	1610	-	1152	-
3f	-	-	3035	1604	-	1154	-
3g	-	-	3064	1612	-	1151	-

Percentages of DPPH radical scavenging activity was tabulated in table 3. The *in vitro* scavenging assay of DPPH radicals was performed spectrophotometrically [19] with ascorbic acid as positive control (Figure 1). The percentage scavenging effects of the compound **3b** at 100, 150, 200 µg mL^{-1} are 57.1, 64.8, 78.1 and compound **3d** at 100, 150, 200 µg mL^{-1} are 51.1, 60.7, 71.4, respectively (Fig.1). The percentage inhibition of the compound **3c** at 100, 150, 200 µg mL^{-1} are 50.2, 60.4, 70.0 respectively. Ascorbic acid presented a scavenging effect of 98.2 % at the concentration of 200 µg mL^{-1}. The moderate inhibition of **3e** and **3f** showed 64.5 %, 63.8 % and 64.1 % at 200 µg mL^{-1}. Compounds **3a** and **3g** exhibited lower inhibition.

Table-3: DPPH radical scavenging activity of the tested compounds

Compound	Scavenging effect (%)		
	Concentration of the tested compounds (µg/ml)		
	100	150	200
3a	30.1	40.5	48.8
3b	57.1	64.8	78.1
3c	50.2	60.4	70.0
3d	51.1	60.7	71.4
3e	42.2	54.8	64.5
3f	42.2	53.0	64.1
3g	30.2	40.0	48.3
Ascorbic acid	73.0	85.3	98.2

Electron donating hydroxyl group in **3b** and **3d** showed more antioxidant activity[20]. The hydroxyl group in **3b** produces enhanced activity probably by *o*-position compared to the *p*-position in **3d**. This indicates the positional requirement of hydroxy group on phenyl ring for enhanced activity. The compound **3c** showed good radical inhibition activity due to the presence of hydroxyl group and methoxy group in the aromatic ring [21,22]. Compound **3g** bear a methoxy groups, **3e** bearing an electron donating ethoxy group

at para position showed similar antioxidant activity. The aromatic ring system with halogens in **3a** was found to be less active than **3g**.

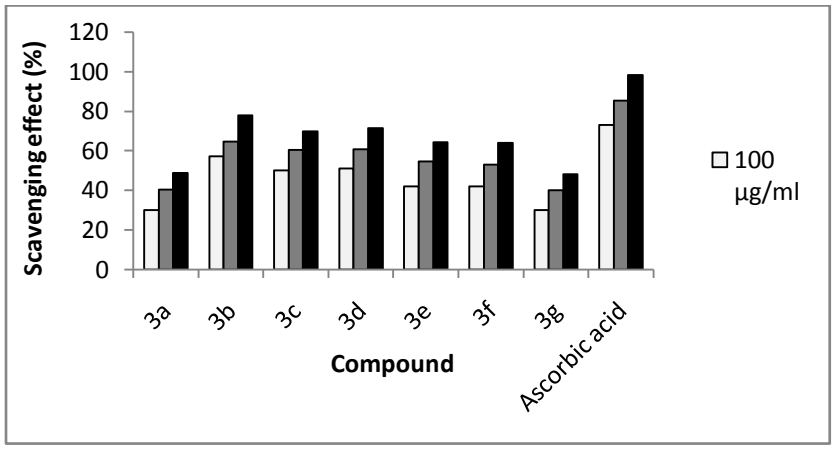


Fig. 1

CONCLUSIONS

In conclusion, a series of new 3,5-dimethoxyaniline derivatives **3a-g** were prepared in good yield, characterized by different spectral studies and their antioxidant activity have been evaluated. Compounds **3b**, **3c** and **3d** demonstrated good antioxidant activity. The structural activity relationship studies reveal that, the substituents on phenyl ring are responsible for antioxidant activity. On the basis of their activity, these derivatives were identified as viable leads for further studies.

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